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RETINAL STIMULATION BY LIGHT SUBSTITUTION

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Ten years ago the authors first met each other in Stockholm, working in Professor Granit's laboratory, and seven years later in Cambridge were able to combine in some research whose foundations had been laid then. In presenting our results in the following four papers, we should like to express our debt to Ragnar Granit from whom we have learnt so much.

Most of our knowledge as to the nature of the visual process has come from subjective observations upon the human eye. A great objective stride was taken when Hartline (1938) succeeded in obtaining records from a single optic nerve fibre in the frog, and Granit's (1947) modification of the technique has enabled the study to be extended to a wide range of vertebrates including several mammals.

All the eyes studied exhibit adaptation to light and darkness and most of them have two distinct mechanisms: scotopic, which shows the spectral sensitivity of rhodopsin (or porphyropsin in fresh-water fish), and photopic, whose sensitivity is displaced some 50 mμ towards the red end of the spectrum. So much is consistent with the well-known Duplicity theory, and the results are usually interpreted in terms of alternative rod and cone mechanisms. However, in detail the structure of fact is not at all simple, and our knowledge of colour mechanisms far from complete. For although the scotopic dominator curve (when it coincides with the absorption spectrum of rhodopsin) represents fairly obviously the sensitivity of the rhodopsin-containing rods, it is not clear what to think about the photopic dominator. It might represent the action spectrum of a single visual pigment contained by most of the cones, or it might result from the convergence upon the ganglion cell studied of nerves from two or more different kinds of cones, each with its own visual pigment. Many possibilities of rod-cone or cone-cone interaction come to mind, but the histology of the retina gives little encouragement to one's inclination to propose something simple.

Nearly all the measurements of spectral sensitivity made with micro-electrodes have been observations of the absolute threshold; that is, the brightness of the light just sufficient to produce a response when flashed upon the eye

previously dark. This technique has two disadvantages. First, the absolute threshold is enormously dependent upon the state of adaptation of the eye, and this is nearly impossible to maintain at the required steady level throughout a long run of measurements which require the eye to be kept in darkness while threshold test flashes are periodically applied. Secondly, it is hard to know whether the threshold sensitivity curve obtained arises from one or from several different kinds of receptor, and if from several in what way they interact to determine the threshold observed. It was in an attempt to overcome these difficulties that the technique of stimulating by light substitution was developed.

The eye is adapted to a steady monochromatic light of any desired colour and brightness, and this is suddenly replaced by a different light. If the second light were identical with the first, obviously no optic nerve signal could arise, but if the intensity were different though the colour were the same, an 'on/off' response would appear when the intensity change, ΔI, exceeded the value given by the Fechner fraction $\Delta I/I$ for that ganglion cell at that level I of adaptation. Clearly, then, when the adaptation light changes to one of the same wave-length and back, the substitution will only be 'silent' when the intensity difference lies within the Fechner interval.

What is to be expected when the two lights are of different colours? If all the receptors connected to the ganglion cell under observation contain the same visual pigment then any two lights equally absorbed by the pigment will be equivalent as stimuli, and hence their exchange is bound to be 'silent'. For each wave-length, therefore, there will be a particular intensity permitting silent substitution, and this intensity (in quanta/sec) is inversely proportional to the absorption of the visual pigment at that wave-length. If, on the other hand, more than one type of receptor is connected to the ganglion cell, either it will be impossible (in general) to obtain a silent substitution or else there must exist a special organization among the receptors, which this technique can help to elucidate.

A further advantage of the method is that it works at a fixed adaptation level which may be set at any desired value. The measurements ideally never leave this level, and even if the preparation is not fully adapted to it, that will not affect the substitution thresholds. In the present paper the technique is applied to the analysis of responses from single ganglion cells in the excised eye of the frog under various conditions of adaptation.

METHODS

Optics

The aim was to shine upon the frog's exposed retina a small, uniform circular field of selected colour and intensity, and then to substitute a different colour with an intensity which could be accurately adjusted over a good range. It was decided not to effect light substitution by using

a shutter to exclude one light and admit the other, as it was thought possible that the eye might detect and signal the flick of the shutter, which could erroneously be interpreted as the detection of colour change.

The method used was to introduce the two alternative lights upon the same optic path but each plane-polarized at right angles to the other. Then rotation of a polaroid in the common beam from 0 to 90° would change the stimulus entirely from one light to the other, and at any intermediate angle θ the light admitted would be

$$x \sin^2\theta + y \cos^2\theta$$

where x, y are the energies of the two lights. If only one pigment is involved, and x and y are adjusted to have equal absorption A, then at θ the absorption will be

$$A(\sin^2\theta + \cos^2\theta) \equiv A \text{ for all values of } \theta.$$

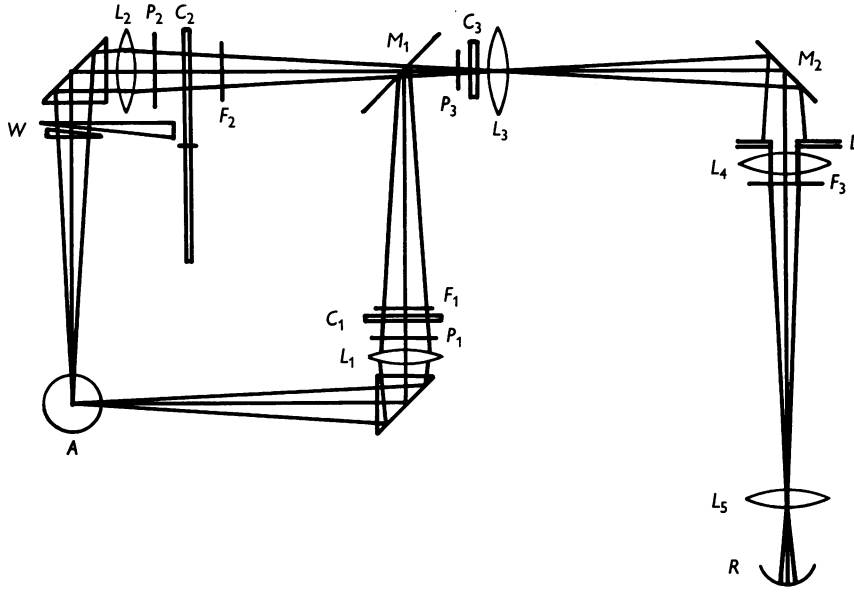


Fig. 1. Diagram of optical arrangement (see text).

Thus the rotating polaroid achieves the condition that if the brightness is matched for the two extremes of the change, the retinal field will be uniform and of this same brightness for every intermediate angle. It was a routine test to obtain the silent substitution when the lights were 'identical'. The fact that this was always possible proved that the change in the plane of polarization itself was not detected by the retina. (It had previously been shown to be undetectable in man by de Vries, 1948, p. 345.)

Figure 1 indicates the optical arrangement employed. The light source was a 6 V car headlamp with a straight vertical filament run from a car battery. The two light paths after reflexion in prisms were united by transmission and reflexion at the thin glass plate M, and the two images of the filament formed upon the lens L_3 by the lenses L_1 and L_2 adjusted to coincide. Now L_1 and L_2 are uniformly illuminated, hence the image of them which L_3 forms upon L_4 constitutes a uniform field which is limited in size by the iris stop D. L_4 is, in effect, the Maxwellian lens for the frog's retina and focuses the image of the filament formed at L_3 upon L_5, which replaces the frog's own lens and projects the uniform field of the stop D upon the exposed retina R.

It is of great importance that in stimulating by substitution nothing should change except the wave-length and the intensity. In particular, the edges of the fields and the direction of incident

light should not alter. This is secured by the use of achromatic lenses, the coincidence of the filament images at L_3 and the fixed limit to the retinal field defined by the stop D, which is projected upon the retina reduced to 1/10 of its linear size.

The light paths 1 and 2 were polarized by polaroids P_1 and P_2 in directions favourable for reflexion and for transmission, respectively, at the glass plate M. The polaroid P_3 in the common beam was mounted in a ball-race; it was arranged so that by pulling a string in opposition to a spring it rotated through 90° and replaced the adaptation beam 1 by the test beam 2, and sprang back upon release.

Twelve interference filters were used to obtain 'monochromatic' lights. They were employed in conjunction with dyed gelatin filters to restrict further the spectral range of transmission. The band widths were all about 10 mμ, and wave-lengths for maximum transmission were, 419, 436, 464, 492, 516, 527, 552, 576, 590, 615, 646, 659 mμ. These filters each with its gelatin corrective were mounted upon a wheel so that any filter pair could be instantly swung into the optic path at C_2. For each individual experiment one filter pair was removed from the wheel and placed in the adapting beam at C_1. When for a particular measurement this filter needed also to be at C_2 it was placed in the common beam at C_3 and the wheel C_2 was turned to present the gap left by the removal of the filter. In this way any colour combination was easily achieved.

Intensity variation was produced by neutral filters F_1, F_2 and by the neutral wedge W which was 15 cm long and with maximum density 3. It was important to introduce a small reversed wedge as compensator, otherwise the retinal field due to this path was not uniform, and consequently in changing to the uniform field of the other path some parts of the retina must inevitably be mismatched. And indeed our attention was drawn to this because no silent substitution was obtained even between what we had supposed were identical fields.

Calibration and plotting

The relative energy of light passed by the various filter pairs in conjunction with the rest of the equipment was measured by receiving the radiation upon a Rb cell calibrated by the National Physical Laboratory. Infra-red was largely excluded by a filter with a sharp cut-off interposed during both the calibration and the subsequent experiment, and all the radiation passed was treated as though it were monochromatic, of wave-length corresponding to the peak transmission.

With the knowledge of this calibration and of the density of the neutral filter and wedge interposed, the relative energy (quanta/sec) may easily be calculated. It is, however, often very valuable to be able to obtain this result at once and to plot the log. spectral-sensitivity curve point by point as measurements are made. It was easy to do this by a simple device. A ruler graduated in millimetres was constrained to move so that its edge kept parallel to the axis of log. sensitivity. The zero of the scale was notched so that it would engage a small nail. Suppose now that the nail were driven into the plotting board at a point whose abscissa represented the wave length of one of the filters and whose ordinate represented the calibrated energy of light through that filter, plotted as minus log. energy upon a scale of 5 cm/log. unit. Then the log. sensitivity measured when x mm of wedge is introduced into the light path is plotted simply by placing a mark on the paper at the point x mm on the ruler whose zero notch engages the nail. It is thus only necessary to drive the 12 nails into the plotting board in the right places, corresponding to the wave-lengths and calibrated energies of the 12 filters, for the log. sensitivity from any wedge setting to be instantly plotted.

Preparation and recording

The experiments were all carried out upon excised, opened frog's eyes from which the front half had been removed by a cut round the equator. The vitreous, which is fluid enough to be absorbed by fragments of blotting paper, was removed to allow freer access for oxygen. The eye was then set up in the apparatus upon a pad moistened with Ringer's solution and connected to the indifferent lead to the amplifier. The eye lay on its back and looked up at the lens L_5 (Fig. 1)

which was held in a horizontal plane by one arm of a micromanipulator. It thus could be accurately moved both vertically to focus the stop D upon the retina, and in the horizontal plane to displace the field across the retina in any direction.

The other arm of the manipulator held the micro-electrode. This was usually a 10 μ Pt wire in glass sealed at the tip, but was sometimes a drawn silver-in-glass electrode (Svaetichin, 1951) with tip diameter of 5–10 μ. There was considerable variation in 'goodness' between one electrode and the next, but little to choose between the two types. The impulses were amplified and led to a cathode-ray tube and loudspeaker in the usual way.

The micro-electrode was brought into contact with the retina and the surface explored for a spot giving good isolated impulses from a single ganglion cell (cf. Barlow, 1953*a*). In some preparations it was difficult to find an active cell, in others the first attempt was successful. All the experiments were carried out on apparently single spikes from ganglion cells, easily distinguished from the smaller and briefer impulses due to the fibres in the optic nerve. We were able to confirm Barlow's (1953*b*) observation that ganglion cells had receptive fields centred at the tip of the electrode, and our fields were usually 0·2–0·5 mm in diameter centred in this way.

The preparation was contained in a small box blackened within, and the experiments were performed in a dark room. The stimulus light was led through a tube 20 cm long and 5 cm wide and stray light was further excluded by covering the box with a black cloth.

RESULTS

Scotopic sensitivity of the human eye

In order to verify that the principle of the method was correct and to check our energy calibrations, some preliminary experiments were performed with the dark-adapted *human* eye. The final lens L_5 (Fig. 1) was shifted and used as an eye-piece through which L_4 was seen by peripheral vision as a large uniform field. The adapting light was a white light of low luminance and this was matched in turn by the various monochromatic lights, the subject himself turning the polaroid P_3 and adjusting the wedge until no change of brightness was seen. It was always possible to match the lights perfectly in this way over a very small intensity range, provided that the intensities used were below the chromatic threshold.

The results plotted as described under Methods are shown in Fig. 2 for the subject K.O.D. (aged 34 years). The dots give, for each wave-length, the scotopic log. sensitivity, and the curve is from Crawford's (1949) authoritative scotopic values for the age-group 30–40 years, plotted upon a quantum basis. The wave-length scale is linear for frequency. The good correspondence confirms our energy calibrations and demonstrates for human rods that the technique is satisfactory and may be used to determine the action spectrum when it is known that only one visual pigment is involved. The method was found to be rapid, convenient and accurate.

Single ganglion of the frog's retina

The discharges observed were fairly slow diphasic spikes of 50–100 μV (see Barlow, 1953*a*), which could be well isolated, recognized throughout the experiment by small characteristics of the individual wave form, and main-

tained under the electrode for several hours in favourable cases. Usually 'on/off' elements were selected with good intensity discrimination so that when changing to a light of the same colour the intensity range over which the substitution was 'silent' was only 0·2–0·05 log. unit. If, however, the new light was of different colour, it was found that the elements behaved in one of two ways. Either the silent interval remained about the same in extent no matter what was the colour change, or it diminished rapidly to zero as the colour difference increased, so that no silent change was possible if the test

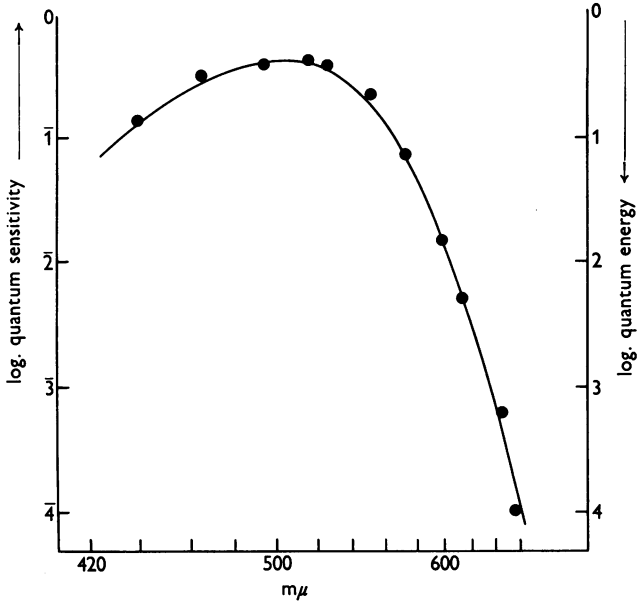


Fig. 2. Human scotopic sensitivity measured by light substitution. Abscissae: wave-length plotted upon a scale linear for frequency. Ordinates: log. quantum sensitivity (on left), log. quantum energy (on right) in arbitrary units. Points represent energies where no change was detectable. The curve is Crawford's (1949) human scotopic sensitivity function for the range at appropriate age group (plotted upon a quantum basis).

light differed much in wave-length from that of the adapting light. Clearly the first type alone is suitable for the investigations of this paper, so in searching for suitable elements those of the second type were rejected. We made no systematic attempt to estimate the relative frequency of the two types, but we have the impression that about 80% of well isolated spikes gave a 'silent' change. Only rarely had the element an identical threshold for 'on' and for 'off'. If 'on' was the lower, then there was a discharge on changing to a brighter light but not upon return (if the brightness difference was near threshold). Likewise there was only a discharge upon return if the comparison light was the dimmer. Thus the silent interval recorded corresponds to the

'on' or the 'off' effect alone, whichever is the more sensitive. This behaviour of the element was very convenient, since during an investigation one knew whether the comparison stimulus was too weak or too strong by noting whether the discharge was at 'change' or at 'return'. Substitutions were repeated at intervals not less than 10 sec, and the comparison field was exposed for about 1 sec. Rapid repetition resulted in 'fatigue' of the intensity discriminating mechanism, so that the brightness difference had to be much increased before a discharge resulted, and in some cases the silent interval could be extended over the range of 1 log. unit as a consequence of rapid trials. With proper precautions, however, the measurements were stable and reliable, as may be appreciated from the results now to be described under three heads.

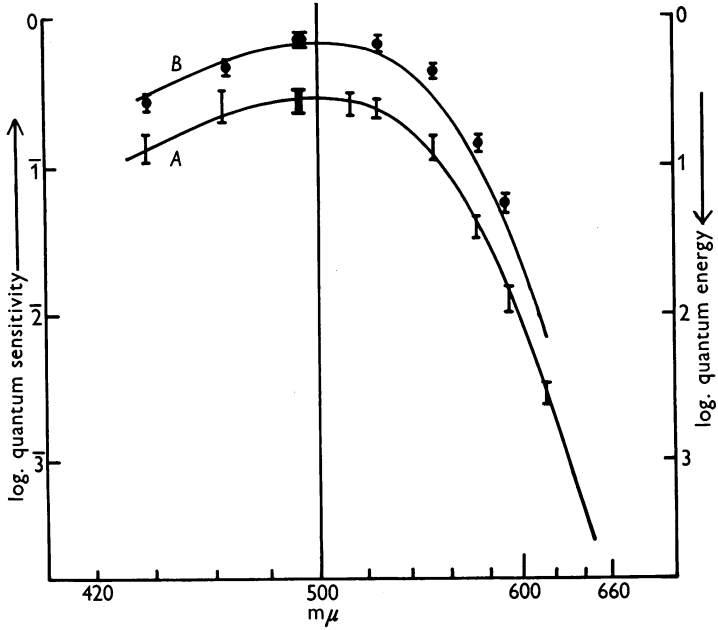
Low-intensity adaptation. The dissection was usually performed in daylight and then the eye was set up and allowed to become dark-adapted for 1–2 hr (Zewi, 1939). The light-substitution measurements were then made by using an adaptation light of 10–50 times the absolute threshold of the element recorded—about 10^3 quanta/mm^2 per second or 0·02 quanta *absorbed* per rod per second ($\lambda = 500$ mμ).

A typical result is shown in Fig. 3*A*, where the thick bar at 492 mμ indicates the adaptation wave-length, and the vertical bars show the width and position of the silent intervals when various wave-lengths were substituted. The vertical length of the thick bar represents the Fechner interval for the element, and if only a single visual pigment were involved all the other bars should be of equal length, and should lie upon the log. absorption curve of the pigment. For in that case a change from one light to another will not alter the rate at which quanta are absorbed by the receptor and hence cannot be detected by it. The curve is the log. absorption curve for a digitonin extract of frog's rhodopsin, plotted from accurate figures kindly sent us by Dr H. J. A. Dartnall. These results are similar to the human measurements (Fig. 2) and confirm the well-known fact that at very low levels of adaptation the spectral sensitivity coincides with the absorption of rhodopsin, and presumably the rhodopsin-containing rods are the only receptors involved. These results were independent of the wave-length of the adapting light.

Figure 3*B* gives the relative sensitivity of the same element as 3*A* when the intensity of the adapting light had been increased 400 times. The curve is plotted displaced vertically for clearness (for obviously the middles of the thick bars which represent 'no change of light' must always coincide). These results illustrate three regularly occurring features. (i) The Fechner interval is reduced (as it is in man). (ii) The sensitivity curve no longer agrees with the absorption of rhodopsin but shows an increased sensitivity towards longer wave-lengths. (iii) But there is still the condition of silent substitution, and the reduced Fechner interval is constant throughout the spectrum.

High-intensity adaptation. When the experiments were performed with the

light-adapted eye, either immediately after dissecting in daylight or after additional light-adaptation of the opened eye with white light of full strength from the apparatus, the characteristic result was as that shown in Fig. 4. The intensity of the coloured adaptation light in these experiments was nearly the maximum available, and 1000–10,000 times stronger than that used for Fig. 3*A*, i.e. about 10^7 quanta/mm^2 per second (λ = 550 mμ).



Figs. 3–6. All these plot wave-lengths horizontally upon a scale linear for frequency and log. quantum energy vertically downwards (on right) or log. quantum sensitivity upwards (on left). The thick bar indicates the wave-length and relative energy of the adaptation light. Its vertical extent shows the 'silent interval' (= ±Fechner fraction). The other vertical lines show silent substitution and indicate the wave-length, the light energy and the intensity at which a silent change is possible.

Fig. 3. *A*, results with adaptation intensity 10–50 times the full dark threshold; the curve is that of rhodopsin absorption (Dartnall). *B*, adaptation intensity 400 times that in *A*; points and curve all displaced upwards for clearness.

Figure 4*A* shows the results of two experiments performed upon a single element; in one the adaptation light was of wave-length 492 mμ (rectangles) and in the other (lines) it was 576 mμ, the intensity being about the same in the two cases. The following features were regularly found. (i) Silent substitution could always be made between any two wave-lengths. (ii) The Fechner interval was constant over the whole curve. (iii) The curve was nearly identical with Granit's (1942) photopic dominator. (iv) There was no selective adaptation, since the curve for silent substitution is identical in shape whether

adaptation is at 492 or 576 mμ. It will be observed that if the photopic dominator involved only a single visual pigment, whose absorption spectrum corresponded to the photopic dominator curve, all the features just mentioned must necessarily follow.

Figure 4*B* gives the results of measuring the photopic dominator on eleven different elements with adapting wave-length 576 mμ in every case. The size of the 'points' in this plot gives the average ± the standard deviations of the

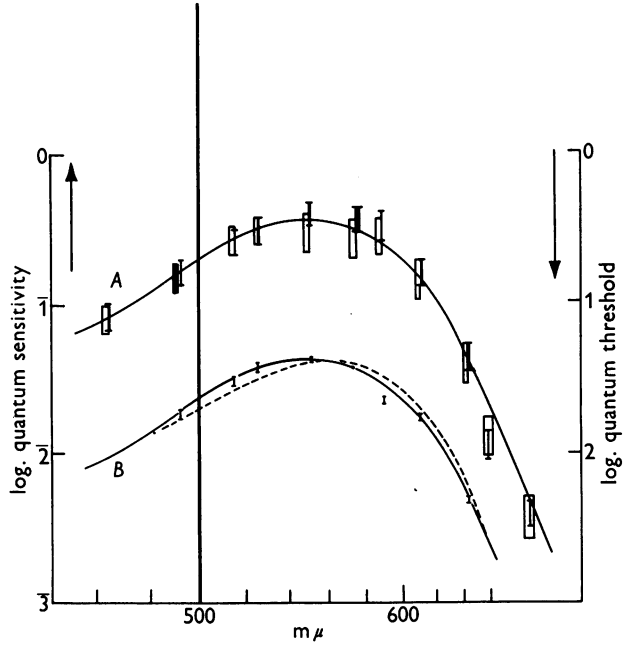


Fig. 4 (see legend Fig. 3). *A*, adaptation intensity 1000–10,000 times that in Fig. 3*A*. Rectangles, adaptation to 492 mμ; lines, adaptation to 576 mμ. The relation does not show any selective adaptation. The curve is our redetermination of Granit's photopic dominator (plotted upon a quantum basis). *B*, mean ± S.D. (11) of photopic silent substitution curves. Interrupted curve, Granit's photopic dominator.

mean for the eleven measurements at each wave-length, a measurement being the wedge reading corresponding to the centre of the silent interval as plotted in Fig. 4*A*.

The dotted curve is Granit's photopic dominator in the frog (Granit, 1942, fig. 9) replotted upon a quantum basis. Our curve (which is also drawn in Fig. 4*A*) seems to show a small but consistent deviation from Granit's. We do not know what to think about our low sensitivity at 590 mμ.

Mesopic adaptation levels. It is convenient to have terms to describe the various states of adaptation seen in the frog's retina. After 2 hr of dark-adaptation the condition shown in Fig. 3*A* is reached, where the spectral

sensitivity corresponds to the rhodopsin absorption, and presumably rhodopsin rods alone are active. This may be called the *scotopic* state. In strong light quite a different spectral sensitivity is found, the photopic dominator (Fig. 4), and this condition is *photopic*.

Between these two levels there exists a wide region of retinal illumination ($10^6 - 10^4$ quanta/mm^2 per second, $\lambda = 500$ mμ) which may be called *mesopic*, where the spectral sensitivity differs significantly from both photopic and

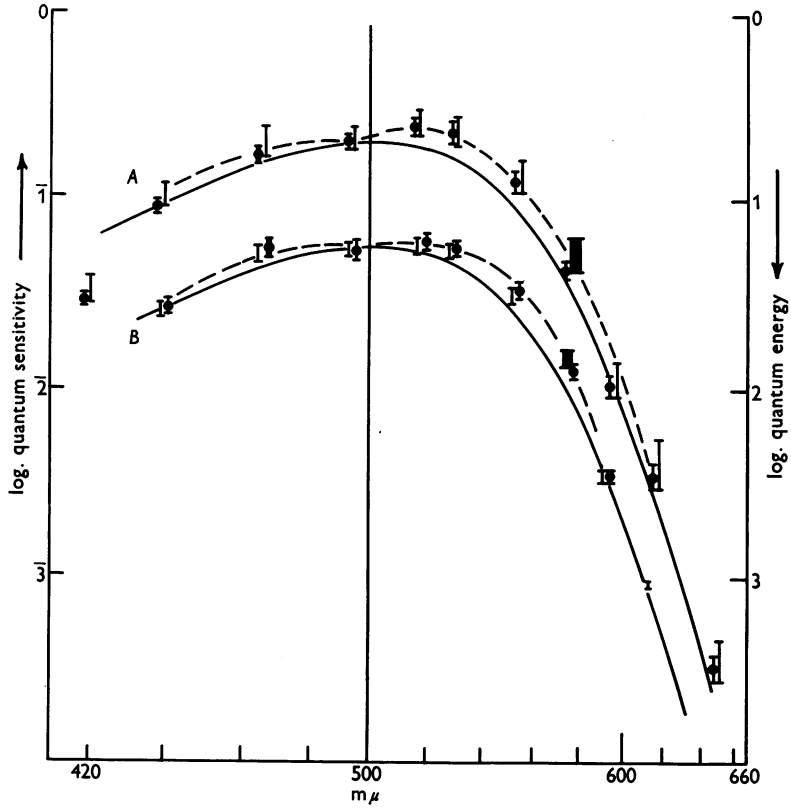


Fig. 5 (see legend Fig. 3). *A*, adaptations to 464 and 576 mμ at intensity levels about that of Fig. 3*B*. *B*, adaptations 25 times as bright as in *A*. Full curve is rhodopsin absorption.

scotopic but lies nearer the latter. An example is seen in Fig. 3*B* showing extra sensitivity in the green, and the curve of Fig. 5*A* exhibits in addition some extra sensitivity in the blue, beyond that expected of rhodopsin (full curve). We are confident that the low value of the 492 mμ point in Fig. 5*A* is no error of determination or of calibration, for the dip here was repeatedly verified in the mesopic state, and was always absent in scotopic determinations.

Figure 5*A* shows in fact the results of two experiments where the same element was tested with adaptation wave-lengths of 464 and 576 mμ adjusted

in intensity to be equally absorbed by rhodopsin. Each curve gives a silent substitution throughout the spectrum and coincides with the other, despite the different wave-lengths to which they were adapted. Figure 5*B* shows the result of repeating the experiments of 5*A* upon the same element, but with the adapting light 25 times brighter, the results being displaced downwards on the figure for clearness, as indicated by the displacement of the rhodopsin curve. The Fechner interval is seen to be smaller in *B* than in *A* but the sensitivity curve appears to be identical.

In the mesopic state, then, as in the scotopic and photopic states, it is possible to obtain a silent substitution between any two wave-lengths if the relative intensities are suitably adjusted. The Fechner fraction remains the same throughout the spectrum, and there is no selective adaptation. But in the mesopic condition a hump of increased excitability above the rhodopsin curve appears always in the green and often in the blue.

We have the impression that as light adaptation is gradually increased above the scotopic level, first the green hump appears and later the blue hump. The appearance is probably gradual over a narrow range after which the hump remains constant. There is no change in humps between Fig. 5*A* and *B*, nor were they ever much more pronounced than this.

Although there appears to be a gradual transition from the scotopic to the mesopic state, with silent substitution possible at every stage, the same is not true for the change from the mesopic to the photopic condition. In Fig. 5*B* at wave-lengths 615 and 438 mμ the Fechner interval is smaller than at intermediate points, and at all wave-lengths outside these no silent substitution could be obtained. This is the beginning of a transformation whose further stages are shown in Fig. 6.

The experiment of Fig. 6 was made upon a different element with adapting light of wave-length 492 mμ. The upper curve was obtained with adapting intensity about the same as that in Fig. 5*B*, which may be called two units of intensity. The next curve is with adaptation at 3 units and it is seen that the range of silent substitution has been reduced. At the level of 6 units it is impossible to obtain silent substitution with any other colour, and the Fechner interval has become small. If adaptation is made still stronger with a bright white light, silent substitution once more becomes possible, but now a new relation has emerged, namely the photopic state, and the sensitivity has changed to the photopic dominator shown in Fig. 4. Thus in the transition from the mesopic to the photopic state something new is taking place, for here alone can we obtain no silent substitution. Below and above this region there appear to be homogeneous but different organizations of receptors: within the transition zone the organization appears heterogeneous.

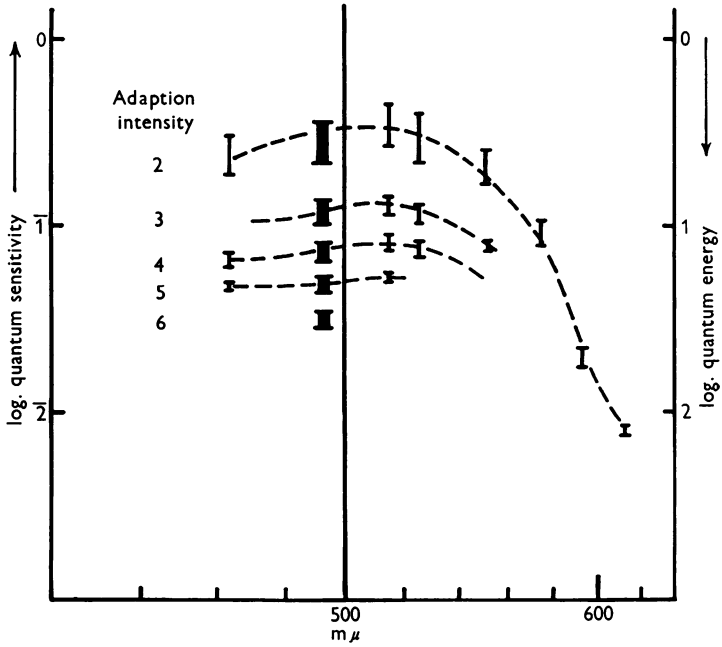


Fig. 6 (see legend Fig. 3). Transition to the photopic state through break-down of silent substitution. Curve 2, adaptation level about equal to that in Fig. 5*B*. Curves 2–6, adaptation gradually increased, as shown by relative intensities. At 6 no silent substitution to any other wavelength is possible. Curves shifted vertically by arbitrary amounts for clearness.

DISCUSSION

The technique of stimulating the frog's eye by changing light from one colour to another has already been studied. Forbes, Burleigh & Neyland (1955) measured the electroretinogram in the excised eye under conditions similar to ours, and particularly studied whether it was possible to make the colour change without generating an electric signal if the intensity ratio of the two lights were suitably adjusted. This they found was always impossible, though changing to an identical light produced no response, showing that the actual transposition of light paths was not the cause of the electrical event.

Bongard (1955) and Bongard & Smirnov (1957) led off responses from single or small groups of ganglion cells and also found that no silent change was possible when one single colour was exchanged for another. But they discovered that any colour could be 'matched' by an appropriate red-blue mixture, so that this substitution did not generate impulses from any of the cells in the small areas studied.

In neither of these investigations were records taken from a single cell selected to exhibit silent substitution, so there is no conflict between the

results of former workers and ours. Our conclusions have the weakness and the strength of selected single-cell analysis: the results refer only to the cell selected, but this cell can be studied in some detail. The technique of light substitution allows some rather stable and accurate sensitivity measurements to be made, and from them the following principal conclusions emerge.

All the ganglion cells studied had quite different spectral sensitivities as between the photopic and the scotopic state, and at a certain transitional level of adaptation it was impossible to make a silent substitution between any two colours. This proves that at that level the ganglion is simultaneously in physiological connexion with two (or more) different types of receptor—presumably both rods and cones.

At the full photopic level (Fig. 4) the simplest assumption consistent with the results of this paper is that a single class of cone is involved, containing a visual pigment whose absorption spectrum corresponds to our photopic dominator curve. Interaction, however, of more than one type of cone is not excluded, but if it occurs it must satisfy a number of special conditions which we shall not discuss until driven to it by further evidence.

At the full scotopic level (Fig. 3*A*) the clear conclusion is that the rhodopsin-containing rods alone contribute to sensitivity. At mesopic levels (Figs. 3*B*, 5) the curve no longer coincides with rhodopsin absorption, but the condition of silent substitution still persists, the Fechner fraction is the same over nearly the whole spectral range and moderate selective adaptation has no differentiating effect.

There are two ways in which spectral sensitivity can alter; either by a change in the nature or organization of the photoreceptors involved (as in the well-known Purkinje shift), or by the interposition or removal of a screening pigment (e.g. coloured spectacles). There is a certain formal simplicity in the supposition that rhodopsin rods alone are active in mesopic conditions but that some change has occurred in a screening pigment, for this would predict the constant Fechner fraction and the absence of selective adaptation which is found. Two screening pigments in the retina have been proposed, one by Dartnall (1948) and one by Wald (1954). Dartnall pointed out that if the yellow (= blue-absorbing) photo-product of rhodopsin bleaching accumulated in the rods it would shift their sensitivity towards the red. This could not account for the observed hump in the blue, and it is not likely to be responsible for that in the green, since the yellow photoproducts do not accumulate in the rods when in contact with the pigment epithelium (Kühne, 1878), and in the lower mesopic range rhodopsin is being bleached only at the rate of about one molecule per rod per second.

Wald suggested that rhodopsin was contained in the rods in compartments which had the following property. Only a compartment containing its full complement of rhodopsin molecules would activate the rod upon absorbing

a quantum of light. Otherwise the light would be absorbed, and rhodopsin bleached as usual, but no contribution to vision would result. From this hypothesis it follows that under steady illumination of moderate intensity those compartments towards the light would tend to remain inactive, since they would generally be without their full molecular complement. So the compartments upon which visual sensitivity depended would be those in the outermost parts of the rods. But these compartments would 'view' the incident light through the whole rhodopsin column of the rod. Consequently the sensitivity will be that of rhodopsin modified by a screening pigment which is rhodopsin itself.

Such a situation could give rise to a sensitivity curve resembling that of Fig. 5, but only if the rhodopsin density was so great that in the scotopic state, where the density is no less, the curve would not correspond in the way it does (Fig. 3) with rhodopsin extinction. Moreover, the self-screening sensitivity curve must be nearly symmetrical about 500 mμ and could not account for the appearance of the green hump alone. The explanation by screening pigments therefore does not seem very satisfactory, and the next paper (Donner & Rushton, 1959) will make it less so.

The alternative explanation of the mesopic state is that both the rhodopsin rods and at least two other types of receptor are involved. Though this in general seems likely enough it will require a quite special kind of interaction between them to account for the possibility of silent substitution, the absence of selective adaptation and the fact that the Fechner fraction is the same throughout the spectrum despite the varying proportions of the different photoreceptors involved. And what is known of the properties of synapses in other parts of the central nervous system would hardly lead us to expect these relations.

We are thus faced with this dilemma. Independent changes in two screening pigments will explain the relations exactly, but there is no independent evidence that adequate pigments exist. Interaction between three types of receptors is plausible, but this would not be expected to result in the observed relations.

The following paper (Donner & Rushton, 1959) will bring evidence of a different kind to show that three types of receptor are in fact involved in the mesopic state, and the fourth paper (Rushton, 1959) will analyse the interaction between them.

SUMMARY

1. An apparatus is described which allows light of one intensity and wave-length to be suddenly substituted for light of another intensity and wave-length.

2. When applied to human scotopic vision the intensity–wave-length relation necessary for the change to be undetected corresponds closely to Crawford's scotopic visibility function (Fig. 2).

3. When applied to excitation of the frog's eye recorded by Granit's technique, silent substitution can be obtained from most ganglion cells in the following circumstances.

4. In full dark-adaptation the sensitivity curve for silent substitution corresponds to the curve of rhodopsin absorption (Fig. 3*A*) or the scotopic visibility curve (as in 2 above).

5. In full light-adaptation the sensitivity curve for silent substitution corresponds to the photopic dominator curve (Fig. 4*A*).

6. At mesopic levels there is still silent substitution but the curve in the green and the blue is more sensitive than is rhodopsin.

7. The transition from mesopic to photopic states is not continuous but passes through a condition where silent substitution is impossible.

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